IN THE SPECIFICATION

Please amend the substitute specification as follows.

On page 5, lines 1-8, please amend the paragraph as follows.

~~FIG. 5 is~~**FIGURES 5A** and **5B** show ~~a photograph of~~ a SDS-PAGE/Western blot of parent strain 35000 and *dsrA* mutant FX517. Outer membranes were prepared, solubilized at 37°C or 100°C and subjected to SDS-PAGE and Coomassie staining (Panel A). For the Western blot (panel B), outer membranes were solubilized at 100°C, transferred to nitrocellulose and probed with anti-DsrA mouse serum. Bound antibody was detected with alkaline phosphatase-conjugated secondary antibody and BCIP/NBT substrate. The asterices indicate the positions of the DsrA protein. STD, molecular weight standards.

On page 30, line 27 through page 31, line 16, please amend the paragraph as follows.

The immunogens of the invention are immunogenic without adjuvant, however adjuvants may increase immunoprotective antibody titers or cell mediated immunity response. Such adjuvants could include, but are not limited to, Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide, aluminum phosphate, aluminum oxide or a composition that consists of a mineral oil, such as ~~Marcol 52~~MARCOL 52, or a vegetable oil and one or more emulsifying agents, dimethyldioctadecyl-ammonium bromide, ~~Adjuvax~~ADJUVAX (Alpha-Beta Technology), Inject Alum (Pierce), Monophosphoryl Lipid A (Ribi Immunochem Research); MPL+ TDM (Ribi Immunochem Research), ~~Titermax~~TITERMAX (CytRx), toxins, toxoids, glycoproteins, lipids, glycolipids, bacterial cell walls, subunits (bacterial or viral), carbohydrate moieties (mono-, di-, tri- tetra-, oligo- and polysaccharide) various

liposome formulations or saponins. Other adjuvants that may be included in vaccine compositions of the present invention include, but are not limited to: surface active substances (e.g., hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyl-dioctadecylammonium bromide), methoxyhexadecylglycerol, pluronic polyols; polyamines (e.g., pyran, dextransulfate, poly IC, carbopol); and peptides (e.g., muramyl dipeptide, dimethylglycine, tuftsin). The immunogen may also be incorporated into liposomes, or conjugated to polysaccharides and/or other polymers for use in a vaccine formulation. Combinations of various adjuvants may be used with the conjugate to prepare the immunogen formulation. Exact formulation of the vaccine compositions will depend on the particular conjugate, the species to be immunized and the route of administration.

On page 20, line 31 through page 21, line 23, please amend the paragraph as follows.

Nucleic acids of the present invention, constructs containing the same and host cells that express the encoded proteins are useful for making proteins of the present invention. Specific initiation signals may also be used to achieve more efficient translation of polynucleotide sequences encoding DsrA. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding DsrA, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature

(Scharf, D. et al. *Results Probl. Cell Differ.* 20,125-162(1994)). In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; ~~Bethesda, Md.~~Manassas, VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

On page 50, lines 12-29, please amend the paragraph as follows.

Western blotting of a variety *H. ducreyi* strains (**Fig. 1**) suggested strongly that DsrA varied in molecular weight and/or amino acid sequence among the strains. Furthermore, we desired to understand whether mutations had occurred in the naturally occurring *dsrA* mutants or whether the possibility of phase variation could account for their inability to express *dsrA*. PCR was used to amplify a 1.2 kb fragment from 8 additional strains, including the *dsrA* mutants (**Fig. 2**, primers 14 and 24). The deduced amino acid sequence indicated that overall the DsrA protein was quite similar between strains (**Fig. 9**; see also **Fig. 10**). Two regions with modest variability were observed and termed variable region 1 and 2 (VR1 and VR2). Variable region 1 included amino acids roughly 90-100 (depending on the strain) and a few substitutions and insertions were noted. Variable region 2 contained either 1, 2, or 3 identical copies of the heptamer repeat sequence NTHNINK (**SEQ ID NO:19**) and spanned amino acids 174--195 in the various strains. It is likely that the different number of repeat sequences was the predominant factor accounting for the variable migration seen in SDS-PAGE and Western blotting. Excepting for mutant strain CIP542(Can), which contained a stop

codon (see below), the sequences for all other 8 DsrA proteins were identical after VR2. Thus, DsrA is highly conserved in sequence, despite its variable mobility in gels.

On page 7, lines 11-29, please amend the paragraph as follows.

~~Dsr-A~~DsrA is an *H. ducreyi* outer membrane protein required for the expression of serum resistance and is encoded by the gene dsrA, described herein. The isolated *H. ducreyi* protein DsrA, and the isolated polynucleotides that encode the protein, are aspects of the present invention. The DsrA protein in its monomer form varies in molecular weight between 28 and 35kDA between different *H. ducreyi* strains in SDS-PAGE and Western blots. The dsrA locus from several *H. ducreyi* strains was sequenced and the deduced amino acid sequences were greater than 85% identical. The major difference between the different strains is found in the amino acid sequence, in which either one, two or three copies of the amino acid sequence NTHNINK (**SEQ ID NO:19**) are present in the VR2 region of the protein ~~present~~; these repeats account for the variability in the monomer form of the DsrA observed in SDS-PAGE. DsrA proteins that contain one, two or three copies of the NTHNINK (**SEQ ID NO:19**) in the VR2 region of the protein, and accordingly having a molecular weight of between 28 and 35 kilodaltons, are all within the scope of the present invention. Additionally, DsrA, as used herein, refers to the amino acid sequences of substantially purified DsrA obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

On page 17, line 23 through page 18, line 12, please amend the paragraph as follows.

Eukaryotic microbes such as yeast cultures may be transformed with suitable protein-encoding vectors. See e.g., U.S. Patent No. 4,745,057. *Saccharomyces*

cerevisiae is the most commonly used among lower eukaryotic host microorganisms, although a number of other strains are commonly available. Yeast vectors may contain an origin of replication from the 2 micron yeast plasmid or ~~anautonomouslyan~~ autonomously replicating sequence (ARS), a promoter, DNA encoding the desired protein, sequences for polyadenylation and transcription termination, and a selection gene. An exemplary plasmid is YRp7, (Stinchcomb et al., *Nature* **282**, 39 (1979); Kingsman et al., *Gene* **7**, 141 (1979); Tschemper et al., *Gene* **10**, 157 (1980)). This plasmid contains the trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics* **85**, 12 (1977)). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Suitable promoting sequences in yeast vectors include the promoters for metallothionein, 3-phospho-glycerate kinase (Hitzeman et al., *J. Biol. Chem.* **255**, 2073 (1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* **7**, 149 (1968); and Holland et al., *Biochemistry* **17**, 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPO Publn. No. 73,657.

On page 33, line 30 through page 34, line 5, please amend the paragraph as follows.

Multivalent live vaccines can be prepared from a single or a few recombinant microorganisms that express different epitopes of *H. ducreyi*. In addition, epitopes of other pathogenic microorganisms can be incorporated into the vaccine. For example, a vaccinia virus can be engineered to contain coding sequences for other epitopes in addition to those of *H. ducreyi*. Such a recombinant virus itself can be used as the

immunogen in a ~~mulivalent~~multivalent vaccine. Alternatively, a mixture of vaccinia or other viruses, each expressing a different gene encoding for different epitopes of outer membrane proteins of ~~H. influenza~~*H. influenzae* and/or epitopes of other disease causing organisms can be formulated as a multivalent vaccine.

On page 34, lines 6-25, please amend the paragraph as follows.

An inactivated virus or bacterial vaccine may be prepared. Inactivated vaccines are "dead" in the sense that their infectivity has been destroyed, usually by chemical treatment (e.g., formaldehyde treatment). Ideally, the infectivity of the virus or bacteria is destroyed without affecting the proteins which carry the immunogenicity of the vector. In order to prepare inactivated vaccines, large ~~quanitites~~quantities of the recombinant vector expressing the desired epitopes are grown in culture to provide the necessary quantity of relevant antigens. A mixture of inactivated viruses or bacteria expressing different epitopes may be used for the formulation of "multivalent" vaccines. In certain instances, these "multivalent" inactivated vaccines may be preferable to a live vaccine formulation because of potential difficulties arising from mutual interference of live viruses administered together. In either case, the inactivated virus or mixture of viruses should be formulated in a suitable adjuvant in order to enhance the immunological response to the antigens. Suitable adjuvants include: surface active substances, e.g., hexadecylamine, octadecyl amino acid esters, octadecylamine, lysolecithin, dimethyl-dioctadecylammonium bromide, N, N-~~dicoctadecyl~~dioctadecyl-N'-N'bis (2-hydroxyethyl-propane diamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines, e.g., pyran, dextransulfate, poly IC, carbopol; peptides, e.g., muramyl dipeptide, dimethylglycine, tuftsin; oil emulsions; and mineral gels, e.g., aluminum hydroxide, aluminum ~~phopshate~~phosphate, etc.

On page 35, lines 18-27, please amend the paragraph as follows.

Bacterial strains used in the experiments described herein are shown below in **Table 1**. For routine growth, *H. ducreyi* was maintained on chocolate agar plates obtained from UNC ~~Hosptial~~Hospital Clinical Microbiology Lab. This medium was prepared using Mueller Hinton base and contained no fetal calf serum. When 5% fetal calf serum was required for optimal growth (*H. ducreyi* strains CHIA and 1157), Gonococcal medium base (GCB) was used for preparation and instructions were followed (Difco). Antibiotics were used at the following concentrations for *E. coli*: ampicillin, 100 μg/ml; chloramphenicol, 30 μg/ml; kanamycin, 30 μg/ml; ~~μg/ml;~~ and streptomycin, 100 μg/ml. For *H. ducreyi,* antibiotics were chloramphenicol, 1 μg/ml or streptomycin, 100 μg/ml.

On page 38, line 30 through page 39, line 3, please amend the paragraph as follows.

The antiserum to DsrA was produced as follows. Outer membranes from *H. ducreyi* strain 35000 were ~~electophoresed~~electrophoresed on large preparative well 12% SDS-PAGE gels. The gel was briefly stained and the corresponding 30 kDa band excised and electroeluted using a Centrilutor (Amicon) following the ~~manufactuers~~manufacturer's instructions. Mice were immunized a total of 3 times with 25 μg of gel purified protein per immunization. Freunds complete adjuvant was used for the first immunization and incomplete for the remainder.

On page 40, lines 6-15, please amend the paragraph as follows.

To obtain sequence downstream of the *dsrA* gene, a third vector-anchored PCR was used (**Fig. 2**, V-A PCR 3). Southern hybridization identified an approximately 4 kb *Bgl* II fragment which hybridized with *dsrA* probes and there are no *Bgl*II sites in the 1.1 kb *Eco*R1 fragment. Fragments of 3-5 kb *Bgl* II restricted chromosomal DNA were isolated and ligated to *Bam*H1, shrimp alkaline phosphatase treated pMCL210 vector.

The ~~ligaton~~ligation reaction was ethanol precipitated and amplified using primers 10 and vector primer T7 (promoter), yielding an approximately 2.5 kb PCR product. The products of all three vector-anchored PCR reactions were sequenced with appropriate primers to obtain preliminary sequence and these sequences confirmed one another (data not shown).

On page 41, lines 9-27, please amend the paragraph as follows.

Plasmid pUNCH 1248 was constructed by PCR. A 900 bp fragment was amplified from *H. ducreyi* strain 35000 using primers 14 and 16 (**FIG. 2**), using the conditions described above for the first two vector anchored PCRs. The product was ligated to pCRII following ~~manufacturers~~the manufacturer's directions, ~~tranformed~~transformed into *E. coli* ~~DH5□~~DH5*α* and recombinants identified by restriction analysis. *E. coli* harboring pUNCH 1248 grew poorly, was propagated only on agar plates to reduce the ~~possibilty~~possibility of mutation/deletion, and gave rise to an occasional larger colony. Subclone 1254 was constructed by isolating the *Eco*R1 fragment of pUNCH 1248 and ligation into *Eco*R1 restricted pLS88. *dsrA* of pUNCH 1254 was mutagenized by insertion of a CAT (Chloramphenicol Acetyl Transferase) into the open reading frame to construct pUNCH 1255. To perform this, a CAT cassette (a *Bgl*II fragment from pNC40 was treated with Klenow to fill-in the ends) was ligated into the *Nde*I site of pUNCH 1254 (previously treated with Klenow to produce blunt ends). pUNCH 1256 was constructed by moving the insert from pUNCH 1255 (containing mutagenized *dsrA*) into plasmid pRSM1791 for subsequent mutagenesis. This was done by isolation of a *Sma*I to *Hin*CII fragment of pUNCH 1255, Klenow treatment and ligation into the *Not*I site of pRSM1791 previously treated with Klenow. Transformation of an *E. coli* host was performed and selection using Amp and Cm yielded pUNCH 1256.

On page 43, lines 3-17, please amend the paragraph as follows.

To rule out that the serum susceptibility of *dsrA* mutant FX517 was due to a mutation elsewhere on the chromosome or polar downstream effects, complementation in trans was performed. Briefly, we PCR amplified the *dsrA* and surrounding locus, using primers 14 and 24 (**Fig. 2**), Klenow treated the PCR product, and restricted the PCR product with *Hin*DIII (which restricts just downstream of *dsrA*, ~~**Fig.2**~~**Fig. 2**). After gel purification, the PCR product was ligated into *Sma*I/*Hin*DIII restricted pLSKS (Wood, G.E. et al., *Target and cell range of the Haemophilus ducreyi hemolysin and its involvement in invasion of human ~~ipithelia~~epithelial cells. Infect and Immun.* **In Press.**) The ligation was ethanol precipitated and *H. ducreyi* strain FX517 electroporated. Streptomycin resistant colonies were screened for production of DsrA by Western blotting and confirmed by restriction analysis. One experimental transformant, pUNCH 1260*dsrA*, and one vector transformant were selected for further study. pUNCH 1260 and the vector pLSKS (negative control) were then electroporated into the three naturally occurring *dsrA* mutants (CIP A75, CIP A77, CIP 542 (Can), Table 1).

On page 43, lines 21-33, please amend the paragraph as follows.

The resistance of *H.ducreyi* to normal human serum was performed as previously described (Odumeru; Carbonetti) with the following modifications: An 18-24 hour culture of *H. ducreyi* from chocolate agar plates was scraped into GCB broth to an OD600 of 0.2. A 10^{-4} to 10^{-5} dilution was made (approximately 1000 CFU/ml, depending on the strain) and ~~aliquotes~~aliquots mixed with pooled fresh normal human serum (NHS) or heat inactivated NHS (~~56C~~56°C, 30 min) to a final concentration of 25 or 50% NHS. After incubation for 45 minutes at ~~35C~~35°C in 5% ~~CO2~~CO_2, 100 ~~ul~~µl ~~aliquotes~~aliquots were plated onto chocolate agar plates and viable counts performed after 48 hours. Data are expressed as percent survival in the fresh NHS as compared to survival in heat-inactivated NHS (number of CFU survivors in fHNS/number of survivors

in heated NHS X 100). Strains containing pUNCH 1260 or pLSKS were propagated and plated on chocolate agar containing streptomycin at 100 μg/ml.

On page 44, line 4 to page 45, line 10, please amend the specification as follows.

During the course of studies characterizing the *H. ducreyi* interaction with PMNs, a series of Western blots ~~were~~was performed using various antibodies to the Opa proteins from *gonococci*. It was found that a polyclonal antiserum to OpaF of gonococcal strain FA1090 reacted at a dilution of 1:5000 with a protein (DsrA) that varied between 28 and 35 kDa in a panel of strains (data not shown). One strain, CIPA75, did not react. CIPA75 was of interest because it had previously been shown to be avirulent in the chilled rabbit model of infection, to be serum susceptible, to exhibit reduced adherence to HEp-2 cells and to have a truncated LOS (Odumeru, J.A. et al, *Role of lipopolysaccharide and complement in susceptibility of Haemophilus ducreyi to human serum.* ~~*Infect*~~*Infect Immun.* **50**,495-9 (1985); Rice, P.A., *Molecular basis for serum resistance in Neisseria gonorrhoeae. Clinical Microbiology Reviews.* **2**, S112-7 (1989). Specific antisera to DsrA ~~was~~were generated using DsrA purified by preparative SDS-PAGE and electroelution of outer membranes from *H. ducreyi* strain 35000. Western blots of several geographically diverse lab and clinical isolates were probed with anti-DsrA (**Fig. 1**). This was done to confirm that the previous cross-reactivity seen with the anti-OpaF serum was due to the presence of DsrA and to ascertain what percentage of strains expressed *dsrA*. The proteins recognized in the DsrA Western blot (**Fig. 1**) and the OpaF Western blot (data not shown) appeared to be identical. Most strains in **Fig. 1[[,]]** expressed an immunoreactive protein, except for the previously reported avirulent strains CIP A75, CIP A77 (25-27) and CIP542 (Can., obtained from Canada) (Alfa, M.J. et al., *Use of tissue culture and animal models to identify virulence-associated traits of Haemophilus ducreyi. Infection & Immunity* **63**:1754-61 (1995)). In contrast, virulent CIP 542 (CDC), obtained from the CDC and previously shown to cause a laboratory acquired infection (Trees, D.L. et al., *Laboratory-acquired infection*

with Haemophilus ducreyi type strain CIP 542. Med Microbiol. 330-337 (1992)), expressed *dsrA*. Previous studies documented that virulent *H. ducreyi* strains are serum resistant. We performed serum ~~susceptiblity~~susceptibility studies of selected *H. ducreyi* strains which did and did not express *dsrA* and these results are summarized at the bottom of **Fig 1**. For the purposes of this study, we arbitrarily termed a strain serum resistant if there were more than 10% survivors when exposed to 50% fNHS serum as compared to NHS. The specific percent survivors (+/- sd) for each of the strains tested in Figure 1 are: 35000, 79%; CIP A75; CIP A77; CIP 542 (Can); CIP 542 (CDC); CHIA; V-1157; M90-02; and 406. Thus, in these initial studies there was a correlation between strains tested which expressed detectable *dsrA* and serum resistance. This correlation between the lack of expression of *dsrA* and serum susceptibility in the *dsrA* mutant strains, some of which also had LOS alterations, could merely be coincidental. Therefore additional molecular studies were performed, culminating in the generation of an isogenic *dsrA* mutant for biological studies.

On page 46, lines 19-31, please amend the paragraph as follows.

The *dsrA* ORF predicted a protein of 28215 daltons, which when processed would yield a mature protein of 26375 daltons. This is in agreement with migration in SDS-PAGE for strain 35000 (**FIG. 1**). Comparison of the deduced amino acid sequence of DsrA with the N-terminal amino acid sequence revealed identity in 28 of 30 amino acids. The first two residues of the mature protein, QQ, were unusual in their charges; however, certain versions of mature YadA begin with two charged amino acids (see below). Just preceding the DsrA QQ residues was the unusual signal peptidase I cleave site of TMA. Consistent with the outer membrane localization, DsrA contained a carboxyl terminal motif ending with a phenylalanine which is found in the majority of integral outer membrane proteins (Struyve, M. et al., *Carboxyl-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. J. Mol. Biol.* **218**, 141-148 (1991)). The mature ~~Drs~~ADsrA protein was predicted to be very basic,